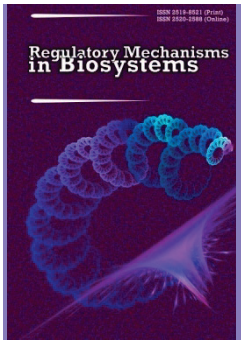

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Screening the possible effect of a phytofabricated nanoselenium-composite from *Eruca sativa* extract in reducing infertility in males

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The antifertility effects of ketoconazole can be avoided or diminished by administering nano-selenium-based-antioxidant plant extract simultaneously or sequentially. Using selenium as nanoparticles (SeNPs) is one of the essential methods for enhancing its therapeutic effects and lowering toxicities. This study aimed to analyze the changes made to the parameters of androgens, such as testosterone and gonadotropin hormones: luteinizing hormone and follicle stimulating hormones together with sperm indexes after administration of antioxidant phytofabricated nanoselenium to mitigate oxidative damage brought on by ketoconazole. In brief, 1% weight-per-volume of the extract was loaded into a solution of 10 mM sodium selenite in various ratios on a magnetic stirrer (50 °C, PH 9) in the dark for 12 hours, left for 48 hours and then sent for characterization, which was performed using ultraviolet-visible spectroscopy (UV-vis spectra), Fourier transform infrared spectroscopy (FTIR), and dynamic light scattering (DLS). Then after the selection of the solution containing the optimal fabricated selenium nanoparticles it was administered to three groups out of seven groups of albino rats with eight animals in each one as follows: Gr. A negative control (no treatment), Gr. B oral ketoconazole 50 mg/kg for fourteen days, Gr. BC, BD, BE1, BE2 and BE3; each one received oral ketoconazole 50 mg/kg for fourteen days followed by: 200 mg/kg *Eruca sativa* (Gr. BC), 0.5 mg/kg oral sodium selenite (Gr. BD), 0.5 mg/kg/cm^2 skin area local nanoselenium (Gr. BE1), 0.25 mg/kg oral nanoselenium (Gr. BE2) and 0.5 mg/kg oral nano selenium (Gr. BE3) respectively for 28 days. After this period, the animals were anesthetized, and plasma testosterone, luteinizing, and follicle stimulating hormones were assessed using Elisa Kit; after that, they were euthanized, and the epididymis of the right testis was carefully removed for evaluation of sperm indices (count, viability, abnormality, and motility). The reduction of selenium ions into PF-SeNPs induced by *Eruca sativa* extracts at a ratio of (1:2) (Na_2SeO_3: *Eruca sativa*) solution was confirmed by the gradual conversion of colour from dark brown to light yellow and then to reddish-orange after the addition of acidic sodium selenite solution and reacting for 12 h. The final reddish-orange colour is the most significant property of nanoparticles. In UV-vis spectroscopy, a strong absorption peak appeared between 268–964 nm with maxima at 268 nm, confirming the formation of nanoselenium. The optimal phytofabricated nanoselenium particles were obtained with a spherical shape, highly stable, and the smallest in size (39.4 nm in diameter) as proved by DLS with Poly Dispersity Index of 0.242 and zeta potential value of –56.57 mV. In the current study, testicles were damaged by administering ketoconazole at a dose of 50 mg/kg/day orally for 14 days. This testicular damage was linked to significant reductions in testosterone levels, elevated levels of LH and FSH, and significant decreases in sperm count, motility, and viability, which in turn affected spermatogenesis. Concurrently, the administration of *Eruca sativa* extract, sodium selenite and nanoselenium solution (in different doses and routes) following ketoconazole was shown to significantly improve biochemical parameters. These improvements included an increase in testosterone levels with little to no impact on LH and FSH levels as well as improved sperm indices. Additionally, the oral nanoselenium groups in 0.25 and 0.50 mg/kg produced the best outcomes with only minor differences between them. In conclusion, the antioxidant effects of the phytofabricated nanoselenium-based *Eruca sativa* leaf extract considerably improved testicular tissues.

Keywords: selenium; nanoselenium; testosterone; luteinizing hormone; follicle-stimulating hormone; sperm indices.

Introduction

Selenium is integrated in selenoproteins that are high in selenocysteine and is one of the most important trace elements in a number of important metabolic processes. It serves as a cofactor for several enzymes that perform oxidoreductase functions, including glutathione peroxidase and thioredoxin reductase (Alsalman et al., 2018). These enzymes have cytoprotective properties, and their activation promotes fertility, lessens inflammatory responses, and acts as a chemo preventive against many malignancies (Landis-Piwowar & Iyer, 2014). In this regard, free radical-induced oxidative stress is thought to be the most significant risk factor that threatens the testicular function. Therefore, an antioxidant defense system, represented by enzymes included in selenoproteins, can combat these free radicals or prevent their formation in the testicular cells (Assumaidaee et al., 2020; Salman et al., 2022). An innovative, expanding field, nanotechnology has significant uses in both science and technology. Nanoparticles are used as a platform for carriers in the pharmaceutical industry to transport medications to their sites of action. It is based on the production of tiny (less than 100 nm) sized particles (Khorana et al., 2019), which improve the biological activity, reduce toxicity, and permit the controlled release of medicines, particularly in capsule form (Patra et al., 2018).

By using chemical or biological reducing agents, metal nanoparticles can be created. At the same time, scientists discovered that utilizing plant extracts with strong antioxidant properties may assist in accomplishing that goal. As the nanoparticle formulation will boost the penetration of selenium and the plant extract into the target region, this may help create a synergistic impact, allowing such formulations to kill two birds with one stone (Khamees, 2021). The antifungal medication ketoconazole has been linked to decreased serum testosterone levels, decreased weight of male

reproductive organs, especially the testes, and decreased epididymis sperm concentration (Amin, 2008) in addition to pathological modifications defined by degenerating Sertoli cells, germ cells and atrophied seminiferous tubules. It is interesting to note that simultaneous or sequential delivery of antioxidant phytochemicals from plant extract could stop such testicular damage (Semet et al., 2017).

The objective of this study was to examine changes in the levels of the androgen like testosterone and gonadotropin hormones: luteinizing hormone and follicle stimulating hormone as well as the sperm indices after the administration of the antioxidant phytofabricated nanoselenium to mitigate the oxidative damage caused by ketoconazole.

Materials and methods

Preparation of selenium nano composite using *Eruca sativa* extract. According to Shareef et al. (2023), 1% w/v of the extract was added to a solution containing 10 mM sodium selenite 0.172 g/100 mL deionized water) at the following ratios: 1:2, 1:14, 1:10, and 1:20 sodium selenite/ extract. The resulting mixture was then stirred for 12 hours in the dark using a hot plate magnetic stirrer at a temperature of 50 °C and a pH of roughly 9, then autoclaved for 15 minutes at 121 °C and 1.5 bar of gas pressure. The autoclaved mixture was then subjected to filter sterilization using a millipore filter membrane (0.22 m) and ultrasonic vibration (20 KHz by a Q700 sonicator) to obtain micron-sized colloidal particles and to ensure an even dispersion of the nanoparticles in the liquid. Finally, characterization of the produced colloid was requested (Shareef et al., 2023).

Characterization of the Nanocomposite:

1) UV-vis Spectra Analysis;

2) Fourier Transform Infrared Spectroscopy (FTIR);

3) Dynamic Light Scattering (DLS).

The samples were analyzed at the Nanotechnology Department of the University of Technology.

Animals and ethical issues. This study was conducted on fifty-six healthy male adult albino rats (age – 102.2 weeks). They were gathered from the animal house at the College of Pharmacy of Al-Nahrin University. The study was approved by the Institutional Review Board (IRB)/College of Medicine/ Baghdad University; all the procedures and experiments were performed following the rules and regulations of the animal ethics committee/College of Pharmacy/Al-Nahrin University.

Grouping of the animals. The experiment was implemented from 1 March 2022 to 31 August 2022. First, the animals were randomly divided into seven groups with eight rats in each, Group A: –ve control, orally administered 2 mL distilled water (DW) daily via gavage tube for 42 days. Group B: +ve control, received 2mL of ketoconazole (KET) solution (50mg/kg/day) orally/day via gavage tube (Anbu et al., 2019) for 14 days, followed by 2 mL distilled water for 28 days. Group BC: received 2 mL of ketoconazole solution (50 mg/kg/day) orally for 14 days, followed by a therapeutic dose of *E. sativa* extract (200 mg/kg/day) orally (Abd-Elsalam et al., 2021) via gavage tube once daily for 28 days. Group BD: received 2 mL of ketoconazole solution (50mg/kg/day) orally for 14 days, followed by a therapeutic dose of sodium selenite solution (0.5 mg/kg/day) orally (Khalaf et al., 2019) via gavage tube once daily for 28 days. Group (BE) nanoselenium group was subdivided into BE1, BE2, and BE3: Groups BE1, BE2, and BE3 received 2 mL of ketoconazole solution (50 mg/kg/day) orally for 14 days, followed by a therapeutic dose of 2 mL nanoselenium solution 0.25 and 0.50 mg/kg/day via gavage tube for 28 days (Groups BE2 and BE3) respectively while BE1 received 0.5 mg/kg/cm^2 skin area local nano emulsion (Kmkm & Ghareeb, 2023) once daily for 28 days. After forty-two days, the rats were ethically anesthetized by chloroform, and blood samples were collected via cardiac puncture in gel tubes and centrifuged at 4000 rpm for 10 min. The serum was drawn and kept at -20 °C until the biochemical studies were performed, which involved measuring FSH, LH, and testosterone serum levels. Soon after euthanization, both testes were excised and weighed, and the epididymis was carefully separated from the right testis; the number, viability, and gross morphology of tail sperm samples in the epididymis were analyzed (Ashidi et al., 2019).

Effect of treatment on androgen and gonadotropin. In this study, plasma levels of testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH) were tested using an ELISA Kit (Enzyme Activity) to assay rat plasma hormonal levels, which are based on the interaction of pre-coated specific antibody on micrelisa strip plate wells with standards or samples.

Sperm parameters. In addition to hormonal assays, sperm parameters were also assessed which include: motility, viability, total sperm count, and morphological abnormalities.

Statistical analysis. The social science statistical software SPSS (IBM SPSS Statistics for Windows, Version 23) was used for the analyses. One-way analysis of variance (ANOVA with Bonferroni correction) was performed to summarize the results, and significance levels of $P < 0.05$ were applied.

Results

Characterization of Nanoselenium. Visual observation. After around 12 hours of adding the acidic sodium selenite solution to the *Eruca sativa* extract, the colour of the extract gradually changed from dark brown to light yellow and eventually reddish orange, indicating the synthesis of SeNPs. This alteration can be attributed to the capacity of the extract to convert selenium ions into SeNPs.

UV-Vis Spectrum Analysis. From 220–1000 nm, the absorption spectrum was measured. There is unmistakable proof that nanoselenium is produced when an intense absorption peak between 268 and 964 nm, with a maximum of 268 nm, is observed (Shareef et al., 2023).

Dynamic Light Scattering (DLS). Particle size measurement. The synthesized Se NPs have an average particle size range of 39.4 to 124.6 nm. The smallest one (39.4 nm with polydispersity index is 0.242) was produced using a (1:2) solution of Na_2SeO_3:*Eruca sativa* (Shareef et al., 2023).

Zeta Potential. In ideal synthesis conditions (1:2). Zeta potential for the created Se NPs was –56.57 mV (Shareef et al., 2023).

Fourier Transform Infrared Spectroscopy (FTIR). The ability of biomolecules and plant extracts to interact with metal ions to form and stabilize selenium nanoparticles was investigated using FTIR spectroscopy. The image in (A) depicts the spectral appearance of an *Eruca sativa* extract devoid of selenium metal. (B) is a representation of the sodium selenite spectrum sample. The spectrum (C) shows the sample that contains selenium metal in *Eruca sativa* extract, presenting the peaks of both the control and test samples, as do the transmission peaks shown in (A) and (B) (Fig. 1). Testosterone level (ng/mL) for the different control and treated groups: Table (1) shows that ketoconazole administered in a dose (50 mg/kg) significantly reduced serum testosterone levels compared to group A. Conversely, all the nanoselenium groups (BE1, BE2, and BE3) showed significantly increased serum testosterone levels compared to group B. Furthermore, a low significant increase in hormone levels was observed in group BE3 in comparison to group BC.

Table 1
Testosterone level (ng/mL) for the different control and treated group

Groups	Testosterone level
Gr. A	5.03 ± 0.14
Gr. B	$4.04 \pm 0.13^{**}$
Gr. BC	4.33 ± 0.14
Gr. BD	4.52 ± 0.15
Gr. BE1	$4.78 \pm 0.17^{€}$
Gr. BE2	$5.03 \pm 0.18^{€€}$
Gr. BE3	$5.18 \pm 0.19^{€€€¥}$

Note: Gr. A and Gr. B represent the negative and positive controls, respectively; meanwhile, each of Gr. BC and BD represents the treatment controls treated with ketoconazole for 14 days, followed by the extract and pure sodium selenite; Gr. BE1, BE2, and BE3 were treated with ketoconazole for 14 days, followed by selenium nanocomposite at topical 0.5 mg/kg and oral 0.25 mg and 0.50 mg/kg, respectively; (*, **, ***), ($^{€}$, $^{€€}$, $^{€€€}$) ($^{¥}$, $^{¥¥}$, $^{¥¥¥}$), ($^{\$}$, $^{\$\$}$, $^{\$\$\$}$), ($^{\wedge}$, $^{\wedge\wedge}$, $^{\wedge\wedge\wedge}$), ($^{\#}$, $^{\#\#}$, $^{\#\#\#}$) is statistically significant difference as compared to groups A, B, BC, BD, BE1, and BE2 with P values $P < 0.05$, < 0.01 and < 0.001, respectively.

Luteinizing hormone (mIU/mL) for the different control and treated groups: normal control LH levels in this research ranged between (3.427–4.353 mIU/mL). Results show a significant increase in LH levels in groups B, BD, and BE2, with the highest value observed in group B (Table 2).

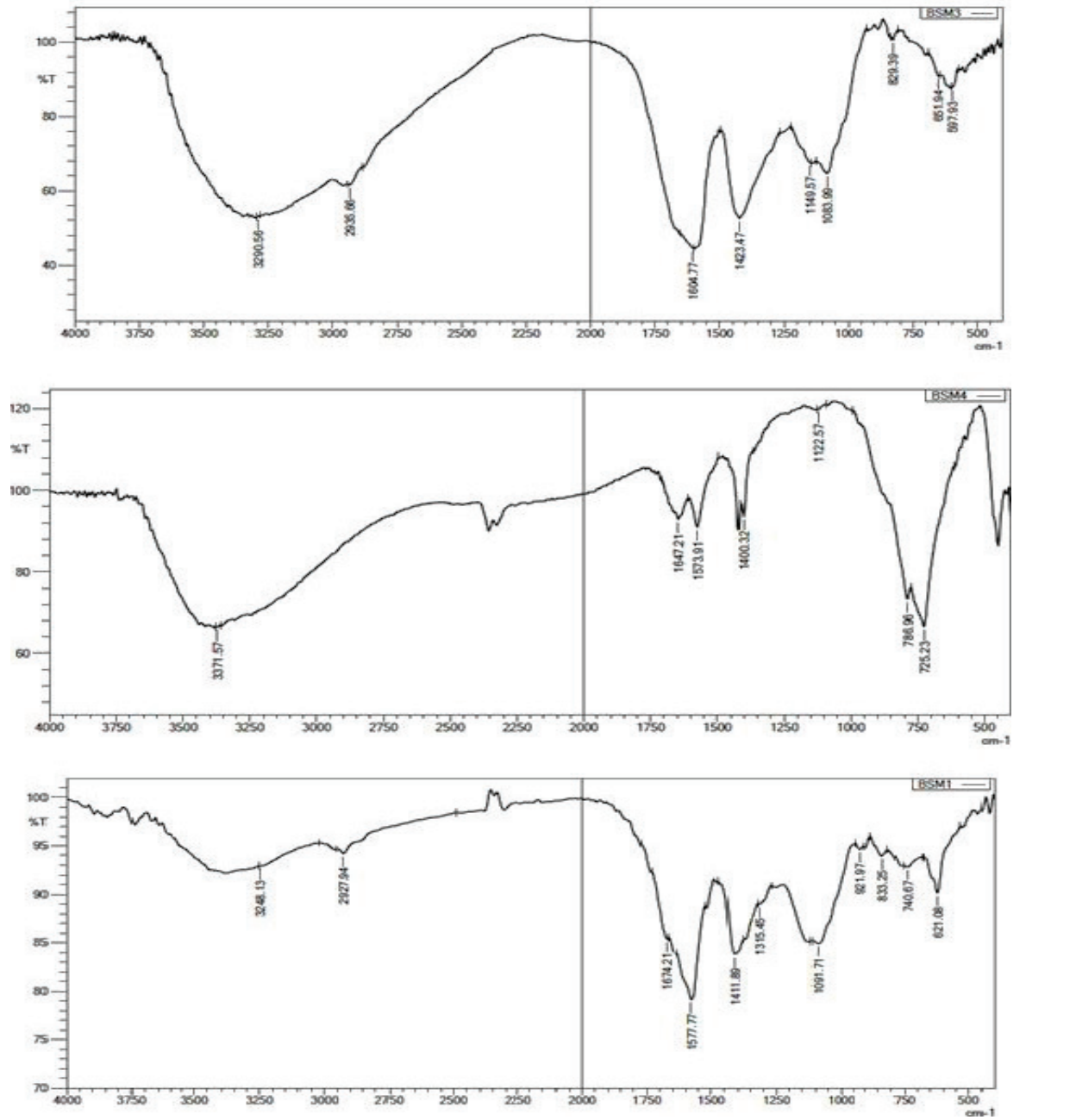

Fig. 1. FTIR spectra of *Eruca sativa* extract, sodium selenite solution, and phytofabricated selenium nanoparticles (PF-SeNPs) (Shareef et al., 2023)

Table 2
Luteinizing hormone for the different control and treated groups

Groups	LH in mIU/mL
Gr. A	3.92 ± 0.22
Gr. B	5.70 ± 0.28**
Gr. BC	4.98 ± 0.24
Gr. BD	5.50 ± 0.28**
Gr. BE1	5.12 ± 0.26
Gr. BE2	5.34 ± 0.22*
Gr. BE3	4.86 ± 0.42

Note: see Table 1.

Follicle-stimulating hormone (mIU/mL) for the different control and treated groups: normal FSH level in Gr. A control group ranged between (4.011–4.943 mIU/mL). Results showed a significant increase in FSH among Gr. B, BD, and BE3 compared to Gr. A (Table 3).

Sperm count. Groups B, BC, BD, and BE1 showed a highly significant decrease in sperm count compared to Group A. At the same time, Group BE2 showed a significant decrease compared to Gr. A, but a highly significant increase in the count in comparison to Gr. B and a significant increase compared to groups BC and BD. At the same time group BE3 exhibited a highly significant increase in sperm count compared to groups B, BC, and BD with non-significant differences with groups A, BE1, and BE2 (Fig. 2).

Table 3
Follicle stimulating hormone (mIU/mL)
for the different control and treated groups

Groups	FSH mIU/mL
Gr. A	4.48 ± 0.21
Gr. B	5.51 ± 0.16*
Gr. BC	5.20 ± 0.16
Gr. BD	5.58 ± 0.19*
Gr. BE1	5.29 ± 0.24
Gr. BE2	5.14 ± 0.26
Gr. BE3	5.55 ± 0.26*

Note: see Table 1.

Sperm viability and abnormalities. Groups B and BC exhibited the highest significant values of dead sperm compared to Gr. A, also there was a significant decrease in the number of dead sperm among groups BC, BD, BE1, BE2, and BE3 in comparison with group B but this did not

reach the control values. Groups B, BC, and BD showed a significant increase in abnormal sperm compared to group A. Groups BC, BD, BE1, BE2, and BE3 showed a significant decrease in the number of abnormal sperm and an increase in normal cells compared to Group B. At the same time, Group BE1 showed significant increase in normal cells and decrease in abnormal ones compared to Gr. BC, while both groups BE2 and BE3 exhibited a significant increase in the normal cells and a decrease in the abnormal cells compared to Group BD (Fig. 3).

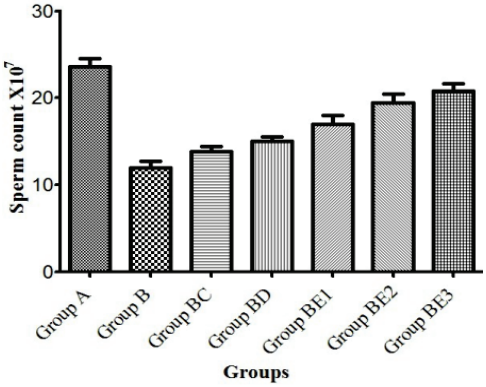

Fig. 2. Sperm count for the different control and treated groups: Group A – negative control, Group B – ketoconazole 50 mg/kg, Group BC – ketoconazole 50 mg/kg followed by *Eruca sativa* extract 200 mg/kg, Group BD – ketoconazole 50 mg/kg followed by sodium selenite 0.5 mg/kg, Group BE1 – ketoconazole 50 mg/kg followed by local nanoselenium 0.5 mg/kg/cm^2 skin area, Group BE2 – ketoconazole 50 mg/kg followed by oral nanoselenium 0.25 mg/kg, Group BE3 – ketoconazole 50 mg/kg followed by oral nanoselenium 0.5 mg/kg

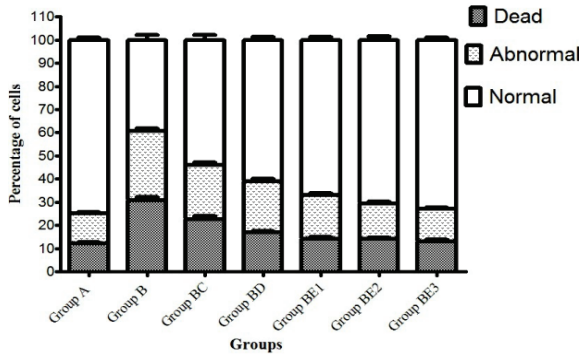

Fig. 3. Sperm viability and abnormalities for the different control and treated groups: see Fig. 2

Sperm motility for the different control and treated groups. Group B exhibited a significant decrease in sperm motility compared to other groups: Groups BD, BE1, BE2, and BE3 showed a significant increase compared to Gr. A. At the same time; there was a significant increase in motility in groups Gr. BC, BD, BE1, BE2, and BE3 in comparison to Gr. B. Furthermore, groups BD, BE1, BE2, and BE3 showed a highly significant increase compared to Group BC (Fig. 4).

Discussion

Testosterone (T) secretion and the gonadotropic hormones LH and FSH are required for normal spermatogenesis (Oduwole et al., 2018). FSH acts directly on the seminiferous tubules, whereas LH indirectly increases steroidogenesis by causing the mitochondrial conversion of cholesterol to pregnenolone and testosterone in Leydig cells. Therefore, a drop in FSH worsens testicular damage, but a rise in T level indicates how much spermatogenesis has been altered and how much spermatogenic cell depletion has occurred (Smith & Walker, 2014).

In the present study, KET-induced reproductive system toxicities, including a decrease in serum T level with increases in both LH & FSH levels (Amin, 2008), indicated testicular damage resulting in hypergonadism. There are numerous causes for this hazardous impact, including: the enzyme $C_{17–20}$ lyase is inhibited by KET, preventing it from converting 17/aS-hydroxyprogesterone to androstenedione (Alizadeh et al., 2018). Another method by which KET-induced testicular toxicity was caused was ROS production and oxidative damage to lipid membranes (Darbandi et al., 2018).

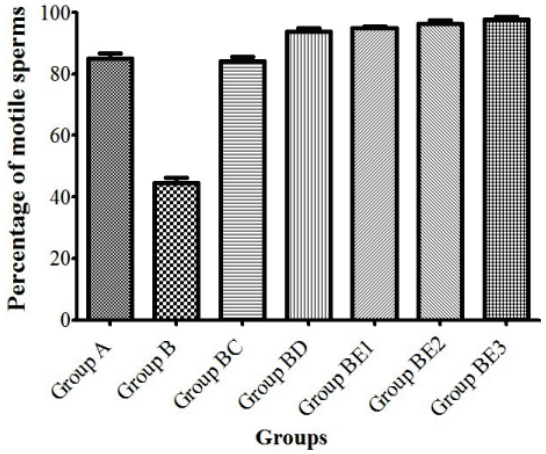

Fig. 4. Sperm motility for the different control and treated groups: see Fig. 2

All PF-SeNPs groups (BE1, BE2 and BE3) retained normal testosterone levels in a dose-dependent manner, especially BE3, which showed the highest T value in contrast to the other treatment groups, which showed significant increment in testosterone level relative to the B group but did not retain normal hormone level as in Group A. These effects, which are probably brought on by PF-SeNPs antioxidant activity and defense of the testicular tissue against oxidative stress and damage, are anticipated to enhance T levels and overall gonadal function (El-Kazaz et al., 2020). Following KET administration for fourteen days, groups BC, BD, BE1, BE2, and BE3 received *Eruca sativa*, sodium selenite, local nanoselenium, and oral nanoselenium 0.25 and 0.50 mg/kg, respectively; LH and FSH levels did not decrease even in the nanoselenium groups, and these results may be ascribed to a number of variables, including the possibility that the supplied dosages of nanoselenium may have been insufficient to exert noticeably decreasing effects on LH and FSH rank (Oduwole et al., 2021). Moreover, interactions with other hormones and individual variability in treatment responses could have contributed to the minimal hormone level changes. The duration of the study's research may also limit the ability to observe more substantial alterations in LH and FSH values (Eendebak et al., 2018).

Regarding sperm indices, in the present study Group B showed a significant increase in dead and abnormal sperm (double head, flattened head, reduced hook or banana head, bent neck, bent tail, and multiple abnormalities were examined) with a significant decrease in normal ones compared to Group A due to KET induced-testicle damage by the various mechanisms mentioned above. At the same time, these findings were re-repaired through the administration of bioactive substances, including *Eruca sativa* leaf extract, sodium selenite, and nanoselenium (in dose and route-dependent manner). Groups BE3, BE2, and BE1 exhibited a highly significant increase in normal cells compared to groups B, BC, and BD with a highly significant decrease in the abnormal number of dead sperm compared to B, BC, and BD. PF-SeNPs tended to exert more increment in sperm indices than micro selenium. These results supported the biochemical hormone parameters mentioned above, which showed positive changes in nanoselenium groups; these results can be attributed to the incredibly small dimensions of the nanoparticles, which enable them to easily pass through biological membranes and accumulate in the blood and other tissues (Hoshyar et al., 2016). Except for Group BE1, which showed negligible variations in the quantity of normal cells in comparison to Group BD. To put it another way, the same dose of oral sodium selenite and local selenium nano emulsion has similar effects on sperm morphology, which indicates that they have similar plasma concentrations. Nano emulsions are widely utilized as drug carriers to improve the absorption of

several medications (Morsi et al., 2017). Additionally, the frequency and overall dosage of the systemic drug were reduced during the therapy period to lessen the negative effects of the medication (Gaber et al., 2023).

Although groups BC and BD showed a significant increase in sperm count compared to Group B, all PF-SeNPs subgroups showed a highly significant boost in sperm count in correlation to Gr. BC and BD, with the highest level observed in BE3 who received oral 0.5 mg/kg PF-SeNPs solution, indicating dose and route-dependent effects. The current results supported escalating therapeutic effects of nano forms in restoring normal cell numbers. These findings were in line with those of Seyedi et al. (2021), who claimed that PF-SeNPs could increase the potential of sperm assembly both qualitatively and quantitatively at an optimal dose (0.5 mg/kg), whereas at higher doses of approximately (1 mg/kg), they could increase spermatotoxicity by disrupting molecular, cellular, and enzymatic pathways. Additionally, the results showed that sperm quality could be negatively impacted by non-optimal dosages of PF-SeNPs (0.1 mg/kg), which can also confuse the testis and induce oxidative stress and sperm DNA damage (Seyedi et al., 2021).

When matched with groups B and BC, highly significant increases in sperm motility were seen in BD, BE1, BE2, and BE3; in addition, BC significantly increased when compared to Gr. B. These holdings will support the beneficial effects of selenium on sperm motility and activity in both its micro- and nanoforms. According to research, sperm motility and viability have been linked to mitochondrial membrane potential (MMP) (Ghafarizadeh et al., 2018). By raising MMP, selenium supplementation protects spermatozoa from mitochondrial harm. In addition to its function as a cofactor to prevent fatty acid oxidation and maintain the integrity of sperm membranes, selenium is also necessary for the GPX enzyme structure. This protects sperm plasma membrane from oxidant damage and the production of MDA on sperm membrane, which improves motility and viability (Dorostkar et al., 2012).

Conclusion

The current research highlights the significance of nanoselenium as a potentially effective treatment agent for enhancing sperm motility, viability, and count, and hence, male fertility. To completely understand the underlying mechanisms and evaluate the long-term effects of nanoselenium treatment on sperm indices and fertility hormones, achieving the goal of respecting male reproductive health, more research is necessary.

Authors declare no conflicts of interest.

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